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THE LARVAL PILOTAXY OF CULEX PIPIENS WITH SPECIAL REFERENCE TO GENETICS

A DISSERTATION SUBMITTED TO THE FACULTY OF THE DIVISION OF THE BIOLOGICAL SCIENCES IN CANDIDACY OF THE DEGREE OF DOCTOR OF PHILOSOPHY

DEPARTMENT OF BACTERIOLOGY AND PARASITOLOGY
1941

By

THOMAS A. HART

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THE LARVAL PILOTAXY OF CULEX PIPIENS WITH SPECIAL REFERENCE TO GENETICS[1]

THOMAS A. HART

INTRODUCTION

Much of the recent epidemiological work on human malaria and *Anopheles* has pointed to a need for more knowledge of the races of mosquitoes involved as well as of the parasites. Although much has been done in morphological, physiological, ecological, and biometric studies on the *Anopheles* mosquitoes, there have been serious drawbacks to genetic research with *Anopheles* because of their eurygamous habits; because individual selections and matings, so important in genetic work, are practically impossible; and because of the expense involved in housing them properly. In order to circumvent some of these difficulties a *Culex* mosquito was chosen for this study. *Culex pipiens* is relatively easy to maintain in the laboratory due to the existence of a stenogamous race in which individual matings after selection can be made. The particular one chosen and used throughout was a stenogamous and autogenous race. Any advances of biological significance made in the study of the species *Culex pipiens* may be related to the general problem of bird malaria. This in turn may serve as a foundation for similar observations within the field of human malaria.

This study was undertaken in order to establish a statistical base line from which comparisons among mosquito races within the species could be made. The morphological feature chosen for study was the pilotaxy of the fourth-stage larva of *Culex pipiens*, and the genetic significance of variations in a particular structure, the numbers of paired tufts on the siphon.

HISTORICAL

Since the beginning of the century there has developed in Europe and in America an interest in mosquito races—their physiological, morphological, and geographical differences. Sergent, in 1903, observed some morphological and physiological differences within the species *Anopheles maculipennis*. However, the first to suggest races within

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this species was Roubaud (1920, 1921), who observed morphological and physiological differences based on the number of maxillary teeth. He concluded that the zoophilic race possessed a greater number of maxillary teeth than did the androphilic race. Wesenburg-Lund (1921) concluded that zoophilism, caused by stall-feeding of cattle, was an environmental rather than a genetic effect. Grassi (1921) demonstrates the presence of Anophelism without malaria as developing from two races within the species (*maculipennis*) only one of which was androphilic. Further research on this subject by Missiroli and Hackett (1927) showed the presence of two definite biological races, one of which was zoophilic, the other indifferent. A later paper by Hackett (1937) reports observations on other species of *Anopheles* which show complexes within the species similar to those of *Anopheles maculipennis*.

Falleroni (1924, 1926) made the initial observations on the egg pattern of *Anopheles*. He found definite color distinctions which later showed racial cleavage into two definite forms. Based on Falleroni's studies were further observations by Hackett and Missiroli (1935) on morphological differences in egg pattern and color, on the hair arrangement of the second abdominal segment of the larvae, and on the shape of the ventral harpaginal spine of the male hypopygium. Finally Hackett (1937) demonstrated eight or nine distinct forms, in the species, based on egg pattern and color. At least seven of these races (Bates and Hackett, 1939) have been well established biometrically, genetically, and physiologically.

In studying the morphology of the adult, Van Thiel (1926, 1927) agreed with Swellengrebel (1924) as to the taxonomic importance of the long- and short-winged varieties.

The race question in *Anopheles maculipennis* is summed up by Bates (1940). He proposed that because of morphological and physiological differences the *maculipennis* populations be classified into species rather than races.

Shortly after work was begun on the study of races of anophelines observations on culicines were also initiated. In 1912 Neumann recorded oviposition in *Culex pipiens* without a blood meal. Huff (1929) in studying ovulation requirements for *Culex pipiens*, confirmed Neumann's earlier findings. However, a race not requiring a blood meal was not distinguished. Grassi (1923) and Legendre (1931a, 1931b, 1932) noted the occurrence of races within this species.

The first definite biological races of this species to be distinguished were those demonstrated by Roubaud and his associates in France (1929, 1930, 1930a, 1931, 1931a, 1933, 1934; Roubaud and Toumanoff 1930, 1930a; and Roubaud and Gaschen 1932). One of these races was anautogenous and eurygamous, the other was autogenous and stenogamous. Tate and Vincent (1936) investigating this problem found true biological anautogenous and autogenous races of *Culex pipiens*. By cross matings between the two races they showed the hereditary character of stenogamy and autogeny. Richards (1941) observed that *Culex pipiens* in the eastern United States shows a differentiation similar to that of the European species as regards stenogamy and autogeny.

Further study of these two races led to a description by Roubaud

(1935) of morphological differences in the egg floats. In the same year de Buck (1935), continuing the study of morphological differences, found that the number of teeth (scales) in the comb of the eighth larval segment in the typical race was greater than the number in the autogenous race.

Jobling (1936) found that the color of the autogenous adults was lighter than that of the anautogenous adults. Among the larvae he found the siphon of the autogenous race to be thicker and shorter than that of the anautogenous race; the same was true of the anal gills. The ventral brush of the autogenous race had more branches than that of the anautogenous race.

Huff (1927, 1929, 1930, 1931, 1934, 1935) showed other notable differences within this species. He found individual mosquitoes some of which proved to be susceptible, others to be refractory to bird malaria parasites. In one instance susceptibility proved to be a simple Mendelian recessive. In one experiment on color of the larvae Huff (1929) found that in the crossing of inbred strains of brown and green larvae the green was a Mendelian recessive.

MATERIALS AND METHODS

Male and female adult mosquitoes were kept in gauze-covered lantern globes over moistened cellucotton in a petri dish. Cooked raisins, placed on the gauze, served as food. Periodically canaries were secured to the top of the lantern globes to afford a blood meal for the females. On the third day thereafter these engorged females were placed over water so that they might oviposit. The eggs were removed to pans filled with distilled water. When the first larvae emerged, feeding was begun. The food consisted of a mixture of dried skim milk, yeast, and liver extract. This feeding continued through the fourth larval stage. After the larvae pupated the pupae were removed from the pans and placed in water-filled evaporating dishes under the lantern globes where the pupae became adults.

For studying the living larvae a temporary mount was made by ringing a large rectangular cover glass with paraffin, covering it with another cover glass, and placing the two of them on a slide. This method eliminated pressure on and decreased the activity of the larva which was enclosed with a drop of water inside the temporary mount.

Several methods were tried for killing and preserving the larvae to be studied for pilotaxy. The method adopted was one suggested by Hopkins (1936).

Fourth stage larvae were killed in hot 70 per cent alcohol. They were then put into fermentation tubes in a solution of lactophenol composed of 20 cc. of phenol, 20 cc. of lactic acid, 40 cc. of glycerine, and 20 cc. of distilled water. This method of preservation gave excellent definition of hairs and body structures. Since this preservative tended to soften the larvae the following method was adopted in order to eliminate pressure on the larva under observation. One large oblong cover glass was ringed with paraffin. On this ring a thin layer of vaseline was applied. A larva in a drop of lacto-phenol solution was placed within the ring. A second large oblong cover glass was used to

cover the temporary mount. This mount was then placed on a glass slide, making possible the use of a mechanical stage in observations. The vaseline which held the two cover glasses together allowed them to be turned over for observations of the dorsal and ventral surfaces of the larva.

Since the oblique position of the siphon prevented a perfectly flat view of the dorsal and ventral surfaces of the larva, it was found advantageous to observe the siphon first and then remove it before observing the head, thorax, and segments of the larva. Each larva was put back into the lactophenol solution after observations were made.

OBSERVATIONS

Pilotaxy.—Using the above method a complete study was made of the pilotaxy of one fourth-stage larva. This study included the number, length, grouping, insertion, and branchings of the hairs on the antennae, head, thorax, segments and siphon. When this study was completed, a descriptive outline and a drawing of the sample larva were made to serve as a working guide for observations on one hundred additional larvae comprising a random sample. Based on these observations and the mathematical calculations of the mode for each character studied, a modal larva was described and drawn (figures 1, 2, 3, 4, and 5).

The system used for numbering the hairs followed Martini's (1923) method for the dorsal surface. The low numbers began at the mid-dorsal line (figures 1, 2, 3, 4) and ranged upward toward the lateral edge. Martini (1923) numbered the ventral surface hairs by beginning at the lateral edge with the next number in the series and ranging upward to the mid-dorsal line. The system adopted here differs from Martini's method in the use of the same numbering scheme for the ventral and dorsal surfaces. Since the eighth segment, the anal segment, and the siphon (figure 5) are in a different plane from the rest of the animal, with the surfaces placed laterally, the numbering system does not follow the plan used on the rest of the larva. On the eighth segment the hairs are numbered consecutively from left to right. On the siphon the hairs are numbered from proximal to distal ends. The anal segment has only one numbered hair.

Martini (1923), Root (1924), and Senevet and Abonnenc (1939) drew homologies between hairs of different segments in larvae of both culicine and anopheline mosquitoes. In this study some apparent homologies have been observed. The following hairs (figures 3, 4) of the dorsal surface of segments 2 to 7 seem to be homologous: Hair number 1 appears to be homologous on segments 2 to 7. Hair number 2 and hair number 6 appear to be homologous with corresponding hairs on segments 3 to 6. Hairs number 4 and 7 seem to be homologous with the hairs of the same numbers on segments 2 to 5. On the ventral surface (figures 3, 4) hair number 3 appears homologous to the corresponding hair on segments 3 to 5. On segments 2, 3, 4, 5, and 7 hair number 4 seems homologous from segment to segment. Before these homologies can be definitely established an intensive, comparative study should be made of the various larval stages in this species and of a considerable number of series of species in the genera.

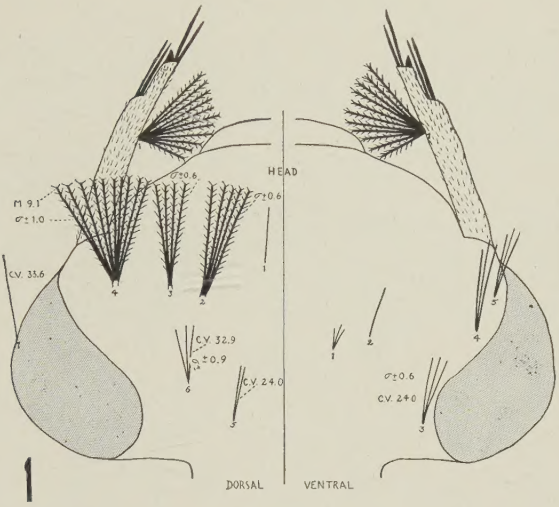

Fig. 1. Pilotaxy of *Culex pipiens* larva. Dorsal view of head.

C. V. Coefficient of variation.
σ. Standard deviation.
M. Mean.

On all the drawings (Figs. 1, 2, 3, 4, and 5) only the upper quartile figures were used for the coefficient of variation and the standard deviation. This accounts for the absence of these figures on most of the hairs. The mean was added to those hairs or hair groups in which it differed from the mode as much as 0.5 or more. The drawings express the modal arrangement of hairs. The number at the base of each hair or hair group follows the system explained in the text.

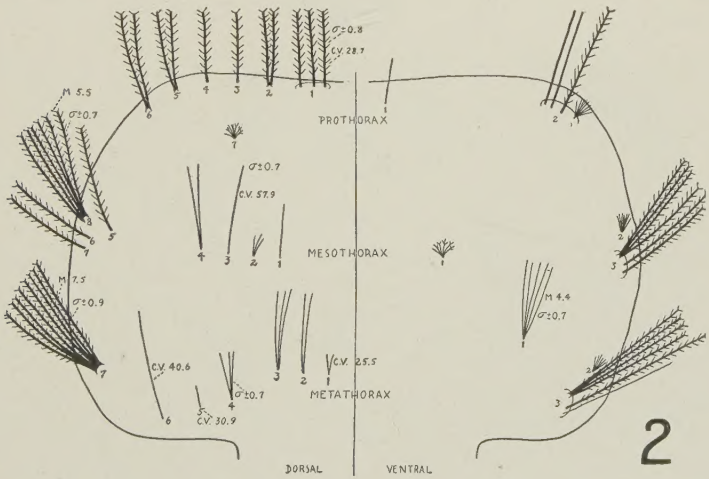

Fig. 2. Pilotaxy of *Culex pipiens*. Thorax, dorsal view. See legend, fig. 1, for terms.

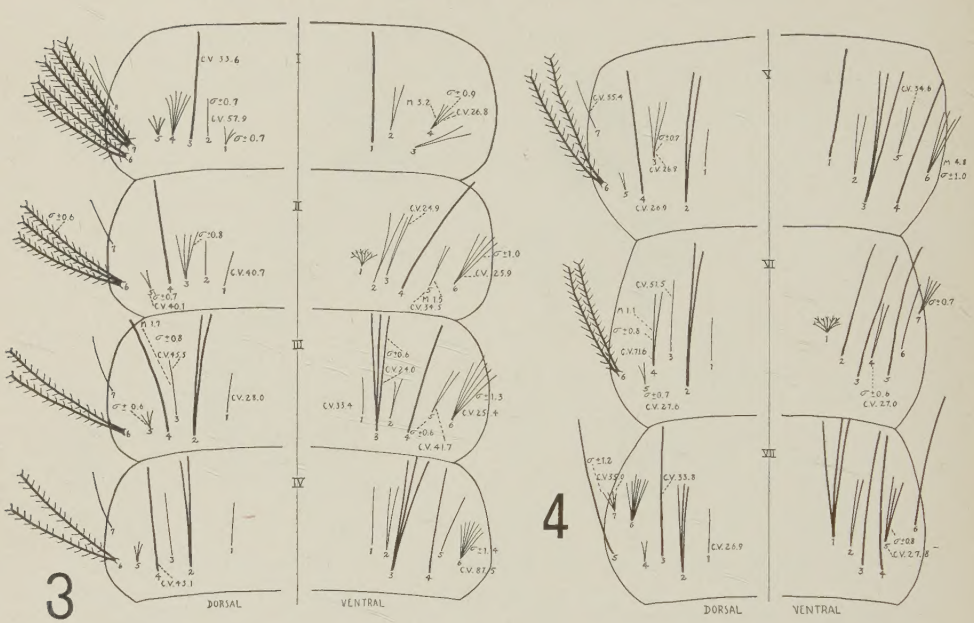

Fig. 3. Pilotaxy of *Culex pipiens*. Abdominal segments I, II, III and IV, dorsal view.
Fig. 4. Pilotaxy of *Culex pipiens*. Abdominal segments V, VI, VII and VIII, dorsal view. (See legend, fig. 1, for terms used.)

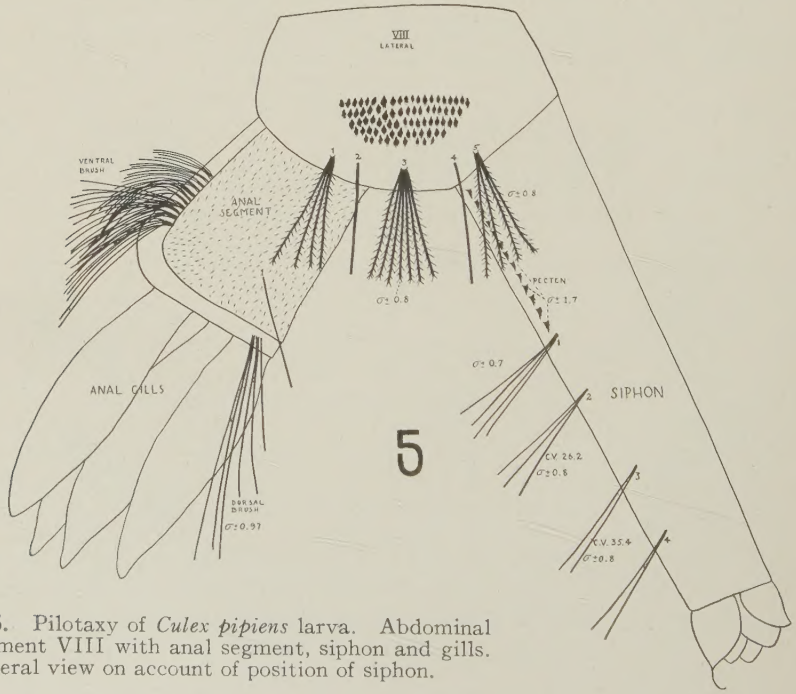

Fig. 5. Pilotaxy of *Culex pipiens* larva. Abdominal segment VIII with anal segment, siphon and gills. Lateral view on account of position of siphon.

STATISTICAL TECHNIQUES

The data from the observations were used to calculate the mean, the standard deviation, the coefficient of variation, and the standard error of the mean for each hair or hair group on the 101 larvae observed.[2] There was a total of 146 different hairs or groups of hairs on the ventral and dorsal surfaces of each larva. Since the animal is bilaterally symmetrical, a total of 292 were observed, and the calculations already mentioned were made for each individual larva. It was found that the total range for the coefficient of variation for all hairs or hair groups was from 0.0 to 87.5 per cent. The total range for the standard deviation was from 0.0 to 1.7. The range of the difference between the mode and the mean was from 0.0 to 0.9. However, on the average, the difference

TABLE I[3]

SELECTED EXAMPLES OF VALUES IN WHICH THE COEFFICIENT OF VARIATION ASSUMES AN UNUSUAL MAGNITUDE DUE TO THE LOW VALUE OF THE MEAN

Hairs	Coefficient of Variation	Standard Deviation	Mean
Dorsum of head—Hair No. 6	32.9	0.9	2.7
Dorsum of mesothorax—Hair No. 3	57.9	0.7	1.7
Dorsum of segment I—Hair No. 2	57.9	0.7	1.1
Dorsum of segment II—Hair No. 5	40.1	0.7	1.7
Dorsum of segment III—Hair No. 3	45.5	0.8	1.7
Ventrad of segment IV—Hair No. 6	87.5	1.4	1.6
Dorsum of segment VI—Hair No. 4	71.6	0.8	1.2
Dorsum of segment VII—Hair No. 7	35.0	1.2	3.6
Siphon:			
Hair No. 2	26.2	0.8	3.2
Hair No. 3	35.4	0.8	2.3

between the mode and the mean was found to be only 5.8 per cent of the mean. Only the upper quartile figures for the coefficient of variation and standard deviation were used on the drawings. In the range of differences between the mode and the mean only those differences of 0.5 or more were added to the drawings.

Since the coefficient of variation is a function of the standard deviation and the mean, it follows that if the standard deviation has a fairly fixed value while the mean changes, the coefficient of variation will change reciprocally. However, since the mean was often close to unity in this study, the changes effected in the coefficient of variation by changes in the mean were of an order greater than is usually seen in ordinary statistical computations. Therefore, while the coefficient of

[2]The complete data for all observations and calculations are being deposited with Prof. C. G. Huff, Department of Bacteriology and Parasitology, University of Chicago.

[3]The figures for this table are taken from data which are being deposited with Prof. C. G. Huff, Department of Bacteriology and Parasitology, University of Chicago.

variation is in this case the more meaningful measure of variability it must be remembered that the means encountered are often of a very low order and consequently the coefficient of variability rises to values higher than would be expected in ordinary statistical material in which the mean is more frequently higher. Table I shows selected cases from the data of this study which illustrate these facts.

COMPARISON OF THE LABORATORY STRAIN WITH A WILD POPULATION

As an initial step in the analysis of the species by biometrical studies of the pilotaxy, a comparison was made between the laboratory population and a wild population with respect to siphonal characters.

TABLE II

COMPARISON OF SIPHONAL HAIR GROUP DISTRIBUTIONS IN THE P_1 GENERATION OF THE LABORATORY STRAIN WITH THOSE OF THE WILD STRAIN

STRAIN	PERCENTAGES OF				TOTAL NO. OF LARVAE
	3 Paired Tufts	4 Paired Tufts	5 Paired Tufts	Unpaired Tufts	
Laboratory....	5.2	63.7	0.0	31.0	1,649
Wild.....	3.0	75.0	2.0	20.0	100
Difference...	2.2 ± 2.2	11.3 ± 4.8	2.0 ± .33	11.0 ± 4.6	

By examining Table II it may be seen that the three-paired tufts, the four-paired tufts, and the unpaired tufts in the two strains do not show clearly significant differences, but in the case of the five-paired tufts the difference is significant.

Observations were made on the number of pecten teeth in the same hundred fourth-stage wild *Culex pipiens* larvae. The same statistical calculations were made for these data as were made for the data on the laboratory strain. The ranges for the number of pecten teeth were from 9 to 17 in both cases. The mode was 13 for both strains. The mean for the laboratory strain was 12.8; the mean for the wild strain was 12.7. The two populations would, therefore, appear to be identical with regard to this character.

The effect of selective breeding on numbers of siphonal hair tufts.— For the selection experiment on the living larvae the siphon was chosen because it was the largest, clearest part of the larvae and the one with the simplest arrangement of hair groups. However, the variation in hair groups was not as large as in some other structures. Technical difficulties were also a factor in determining the choice of the siphon in preference to any other structure.

During previous observations on preserved specimens of *Culex pipiens* fourth-stage larvae it was found that the mode for the arrangement of hair sets on the siphon was four groups bilaterally placed. A number of larvae were found with three bilaterally placed groups of hairs. A few larvae had five bilaterally placed groups of hairs. A large number had asymmetrically arranged hair groups in various combinations, such as four on one side, three on the other; four on one side, five on the other; three on one side, five on the other; three on one side, two on the other. The bilateral arrangement of three hair groups on the siphon was chosen for the selection experiment because the four bilaterally placed groups were the modal arrangement of siphon hairs; because the asymmetrically arranged grouping of hairs was too variable, and not as subject to statistical analysis; and because the number of larvae with five bilaterally placed groups of hairs was too small.

Beginning with the W-stock strain and continuing through the P_1, F_1, F_2, and F_3 generations, selection was made of larvae having siphons with three bilaterally placed groups of hairs. A fourth-stage larva was chosen at random from the W-stock strain. It was placed in a temporary mount and observed under low power. The number of hair groups on the siphon was ascertained, and the larvae with 3 paired hair tufts were segregated. All larvae with 4 paired or unpaired tufts selected from each generation were discarded because in this experiment they were not of any genetic importance. The larvae with 3 paired tufts selected from the W-stock strain constituted the P_1 generation. As these larvae pupated they were transferred to water-filled evaporating dishes under gauze-covered lantern globes. As soon as sufficient numbers of male and female adults had emerged the females were given a blood meal. On the third day thereafter the globes containing the fed females were placed over water so that they might oviposit. The eggs thus obtained were the beginning of the F_1 generation. Each succeeding generation was similarly treated, and the same procedure used in selection of P_1 larvae from the W-stock strain was followed for the F_1, F_2, and F_3 generations. At a point in the selections on the F_3 generation isolation procedures were instituted for egg rafts for the purpose of attempting to establish a homozygous strain. Of the five egg rafts isolated, three were infertile. Selection of larvae which developed from the other two egg rafts showed a distribution of 3 paired, 4 paired, and unpaired tufts not essentially different from those of egg rafts not isolated. As the F_3 larvae pupated they were isolated in test tubes partly filled with distilled water and plugged with cotton. Upon emergence the adults were placed in pairs, one male and one female, in lantern globes. Of the several attempts to induce the females of the isolated pairs to take a blood meal, only one was successful. The eggs from this female were infertile. Isolation of adult pairs in test tubes was tried also but this proved to be no more successful than isolation in lantern globes. Attempts to feed were continued until all the isolated pairs had died. The adults which developed from these select larvae seemed to be weak. Consequently one group of adults died before another group matured. This made it impossible to build up a substantial colony in this generation.

The mass selection experiment on larvae with 3 paired tufts on the siphon began with the W-stock on March 19, 1941. The experiment was terminated August 4, 1941, with the selected inbred F_3 generation. Table III summarizes the results of the experiment.

TABLE III

RESULTS OF MASS SELECTION EXPERIMENT FOR THE THREE PAIRED TUFT CHARACTER ON THE SIPHON OF THE LARVAE

GENERATION	NUMBER OF LARVAE WITH:			TOTAL	PERCENTAGE 3 PAIRED TUFTS
	3 Paired Tufts	4 Paired Tufts	Unpaired Tufts		
P_1	86	1,051	512	1,649	5.2
F_1	150	1,122	750	2,022	7.4
F_2	116	818	576	1,510	7.7
F_3	42	75	86	203	20.7

Male mosquitoes were chosen from the P_1, F_1, F_2, and F_3 generations. These were killed, cleared, and mounted to show genitalia. Each of the males chosen at random proved to be *Culex pipiens*. These observations were made and recorded in order to be certain that the animals with which the study ended were of the same species as those used to begin the study.

DISCUSSION

The data presented in this study and the statistical analysis of these data lay a groundwork for a quantitative analysis of the species by use of clearly visible morphological characters. The statistical data described here have served to establish norms for the laboratory strain of *Culex pipiens* as regards pilotaxy. In general, these norms agree with the findings of systematic culicidologists who arrived at their conclusions through less quantitative methods. This agreement shows that the characters of the larva known to be best for species identification show a low degree of variability. Extensive examination of the pilotaxy of this species is needed. Analysis of a wide-spread species revealing valid, small differences would lead to a more complete understanding of speciation in this group of mosquitoes. The population comparisons made thus far have been in two groups separated by only 10 miles. Although these two groups were very similar in pilotaxy, it is important to note that one significant difference was found in a siphonal character.

In the selection experiment the morphological character used was found to be determined to some degree, at least, by genetic factors. The fact that susceptibility in these mosquitoes has also been established as hereditary opens up the possibility of a correlation between these two characters which might aid in the solution of the more complex problem of malarial epidemiology. Information and techniques discovered in the field of avian malaria could probably then be applied to *Anopheles* and human malaria.

SUMMARY

Observations were made on the pilotaxy of 101 prepared specimens of a stenogamous, autogenous laboratory strain of *Culex pipiens* fourth-stage larvae.

The mode, the mean, standard deviation, and coefficient of variation were calculated for each individual hair or hair group.

A selection experiment, based on siphonal hair groupings, was performed. The non-modal arrangement of three bilaterally placed hair groups was chosen. Selection and inbreeding for this character through four generations showed a continually increasing percentage. These findings indicate that the character is hereditary.

To serve as an initial attempt at an analysis of the pilotaxy of the species as a whole, a comparison was made between the laboratory population and a wild population in respect to 5 pilotaxic characters. In this study it was found that there was a significant difference in one character out of the 5 studied, even though the populations were very close together geographically.

LITERATURE CITED

Bates, M. 1940. The nomenclature and taxonomic status of the mosquitoes of the *Anopheles maculipennis* complex. Annals Entom. Soc. Amer., 33: 343–356.

Bates, M., and **Hackett, L. W.** 1939. The distinguishing characteristics of the populations of *Anopheles maculipennis* found in southern Europe. Verh. VII. Kongr. Entom., Berlin, 1938, 3: 1555–1569.

de Buck, A. 1935. Beitrag zur Rassenfrage bei *Culex pipiens*. Z. Angew. Ent., 22: 242–252.

Falleroni, D. 1924. Studio sugli *A. maculipennis* delle Paludi Pontine. Tipografia Economica (Recanati), (from Hackett, 1937).

1926. Fauna anofelica italiana e suo habitat (paludi, rissaie, canali). Metodidi lotta contro la malaria. Riv. Malariol., 5: 553–593.

Grassi, B. 1921. Razza biologica di anofele che non punge l'uomo. Un singolarisimo caso di anofelismo e paludismo senza malaria. R. C. Accad. Naz. Lincei, ser 5, XXX (2): 30 (2): 11–13.

1923. Razze biologiche differenti di *Culex pipiens*. R. C. Accad. Naz. Lincei, 32: 457–464.

Hackett, L. W., and **Missiroli, A.** 1935. The varieties of *Anopheles maculipennis* and their relation to the distribution of malaria in Europe. Riv. Malariol., 14: 45–109.

Hackett, L. W. 1937. Recent additions to our knowledge of *Anopheles maculipennis* races. Bull. Health Organization of the League of Nations, 6: 1–16.

Hopkins, G. H. E. 1936. *Mosquitoes of the Ethiopian Region*. London: Oxford University Press. 250 pp., 158 figs. (See pp. 26–27.)

Huff, C. G. 1927. Studies on the infectivity of Plasmodia of birds for mosquitoes with special reference to the problem of immunity in the mosquito. Amer. Jour. Hyg., 7: 706-734.

1929a. Ovulation requirements of *Culex pipiens*. Biol. Bull., 56: 347–350.

1929b. Color inheritance in larvae of *Culex pipiens*. Biol. Bull., 57: 172–175.

1929c. The effects of selection upon susceptibility to bird malaria in *Culex pipiens*. Ann. Trop. Med. and Parasit., 23: 427–442.

1930. Individual immunity and susceptibility of *Culex pipiens* to various species of bird malaria as studied by means of double infection feedings. Amer. Jour. Hyg., 12: 424–441.

1931. The inheritance of natural immunity to *Plasmodium cathemerium* in two species of *Culex*. Jour. Prev. Med., 5: 249–259.

1934. Comparative studies on susceptible and insusceptible *Culex pipiens* in relation to infections with *Plasmodium cathemerium* and *P. relictum*. Amer. Jour. Hyg., 19: 123–147.

1935. Natural immunity and susceptibility of culicine mosquitoes to avian malaria. Amer. Jour. Trop. Med., 15: 427–434.

Jobling, B. 1936. Morphological differences in the larval stages, between autogenous and anautogenous races of *Culex pipiens*. Trans. R. Soc. Trop. Med. Hyg., 30: 4.

Legendre, J. 1931a. Le moustique cavernicole ou l'adaptation de *C. pipiens* a l'urbanisme moderne. Bull. Acad. Méd., 106: 86–89.

1931b. Le moustique stercoraire. Bull. Acad. Méd., 106: 276–280.

1932. Androphilie ou zoophilie chez le moustique commun *Culex pipiens*. Bull. Acad. Méd., 107: 317–320.

Martini, E. 1923. Uber einige fur das System bedeutungsvolle Merkmale der Steckmucken. Zool. Jahrb., 46: 517–591.

Missiroli, A., and **Hackett, L. W.** 1927. La regressione della malaria in alcune regioni d'Italia. Riv. Malariol., 6: 193–243.

Neumann, R. O. 1912. Brauchen die Steckmücken zur Reifung ihrer Eier Blut als Nahrung? Arch. Schiffs-u. Tropenhyg., 16: 27–30.

Richards, G. A. 1941. A stenogamic autogenous strain of *Culex pipiens* in North America (Diptera: Culicidae). Entom. News., 52: 211–216.

Root, F. M. 1922. The larvae of American Anopheles mosquitoes in relation to classification and identification. Amer. Jour. Hyg., 2: 379–393.

1924. The larval pilotaxy of *Anopheles quadrimaculatus* and *Anopheles punctipennis*. Amer. Jour. Hyg., 4: 710–724.

Roubaud, E. 1920. Les conditions de nutrition des Anophèles en France *A. maculipennis* et le rôle du bétail dans la prophylaxie du paludisme. Ann. de l'Inst. Pasteur, 34: 181–228.

1921. La différenciation des races zootrophiques a'anopheles et le régression spontanée du paludisme. Bull. Soc. Path. Exot., 14: 577–601.

1929. Cycle autogène d'attente et générations hivernales suractives inapparentes chez le moustique commun *Culex pipiens*. C. R. Acad. Sci., 188: 735–738.

1930a. Suspension évolutive et hivernation larvaire obligatoire provoquées par la chaleur, chez le moustique commun, *Culex pipiens*. Les diapauses vraies et le pseudodiapauses chez les insectes. C. R. Acad. Sci., 190: 324–326.

1930b. Sur l'existence de races biologiques génétiquement distinctes chez le moustique commun *Culex pipiens*. C. R. Acad. Sci., 191: 1386–1388.

1931a. Sur l'autogénèse chez *Culex pipiens*. Bull. Soc. Path. Exot., 24: 384–387.

1931b. Les phenomènes d'histolyse larvaire postnymphale et d'alimentation imaginale autotrophe chez le moustique commun *Culex pipiens*. C. R. Acad. Sci., 194: 389–391.

1933. Essai synthetique sur la vie du moustique commun (*Culex pipiens*). L'évolution humaine et les adaptations biologiques du moustique. Ann. Sci. Nat., 16: 5–168.

1934. Au sujet des différenciations raciales chez le moustique commun *Culex pipiens*. Ann. Parasit. Hum. Comp., 12: 337–339.

1935. La microstructure du flotteur de l'oeuf dans les races biologiques de *Culex pipiens*. Bull. Soc. Path. Exot., 28: 443–445.

Roubaud, E., and **Gaschen, H.** 1932. Différenciation des races biologiques de *Culex pipiens* par l'adaptation larvaire aux milieux ammoniacaux. Bull. Soc. Path. Exot., 25: 1053–1058.

Roubaud, E., and **Toumanoff, C.** 1930. Sur une race physiologique suractive du moustique commun *Culex pipiens*. Bull. Soc. Path. Exot., 23: 196–201.

Senevet, G., and **Abonnenc,** E. 1939. Moustiques de la Guyane francaise. II. Le genre *Culex*. Algiers. Archives de L'Institut Pasteur, 17: (No. 1), 62–134.

Sergent, Ed., and **Sergent, Et.** 1903. Observations sur les moustiques des environs d'Alger. Ann. Inst. Pasteur, 17: 60–67.

Swellengrebel, N. H. 1924. Nadere byzonderheiden over de herfst-en winterbes. Metting met malaria parasieten van *A. maculipennis* om Amsterdam. Nederl-Tydschr. v. Gensesk., 68: 750.

Tate, P., and **Vincent, M.** 1936. The biology of autogenous and anautogenous races of *Culex pipiens*. Parasitology, 28: 115–145.

Van Thiel, P. H. 1926. Maxillenzahnzahl und Flügellänge bei *A. maculipennis*. Beih. Arch. f. Schiffs-u. Tropenhyg., 30: 585–594.

1927. Sur l'origine des variations de taille de l' *A. maculipennis* dans les Pays-Bas. Bull. Soc. Path. Exot., 20: 366–390.

Wesenberg-Lund, C. 1921. Les anophélines du Danemark et les fièvres paludéennes. C. R. Soc. Biol., 85: 386.